ON THE STABLE COLOURS OF THE RETINA.

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(Pl. III. IV. V.)

We have chosen the name "stable" for some of the colours of the retina here to be described, not in order to imply that they completely resist the action of light, but simply to place them in opposition to that pigment of the retina which alone, as far as is known up to the present time, is in the highest degree sensitive to light, namely "visual-purple." It is so universally the case that pigments which give rise to colours by absorption, and to which the retention of particular waves of light is peculiar, are at the same time altered and decomposed by light, that it is difficult to find absolutely stable colours either in nature or in manufactures.

It must be an important reason, well worthy of the attention of science, which urges art and industry to seek unceasingly for durable, light-resisting colours among those compounds which are also permanent at high temperatures, and in cases where mineral colours cannot be employed, to give the preference among the numerous coloured carbon-compounds at our disposal to those which endure heating to a relatively high degree without decomposing, rather than to those which are more brilliant in hue. The colouring substances of indigo

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and madder which for the most part sublime without change even at high temperatures may be mentioned as examples of this latter class; in the case of these, however, it is probable (and in many cases certain) that they fade away under circumstances which are in themselves innocuous, if the action of light be superadded. We have found Vogel's statement* perfectly true, that purpurin is not bleached in the dark in presence of excess of alkali, but certainly is in the light. In general therefore there is little to be said against the principle that only mineral colours are stable and the so-called organic colouring matters unstable. Yet it must be remarked that there are among the latter, some belonging exclusively to the animal body which far surpass in stability those used for dyeing. These are the colouring-matters of the blood, especially reduced hæmoglobin, in which we were unable to detect any change at all under the action of light; also the black or brown pigments of various origin.

Hoppe-Seyler† a short time ago justly drew attention to the extraordinary stability of reduced hæmoglobin. This I can confirm and extend with reference to the action of light. I sealed up aqueous solutions of the purest possible hæmoglobin in glass tubes, together with pulverulent iron, reduced by hydrogen, and an extremely small bubble of air; these I have kept for nine years, and in the course of last year I exposed them to light and to the action of the sun, without observing any change perceptible by the spectroscope or other means. In several of these tubes, which for many years I have been accustomed in my lectures to show and to place before the spectroscope, were solutions which from the beginning were so dilute that the single band of reduced hæmoglobin could be just easily recognised; this same band can be seen at the present time with the same distinctness. How oxyhæmoglobin behaves when exposed for a lengthy period to light, could not be determined, since the solution cannot be kept without acquiring the properties of the so-called methæmoglobin, or of that substance which has been designated of late as peroxyhæmoglobin, and finally undergoing complete decomposition even without the action of light.

With regard to the so-called peroxyhæmoglobin, I am able to confirm the various modes of its formation, discovered first by Gamgee and of late especially by Jäderholm. I believe, however, with Hoppe-Seyler, that we have not to deal in this case with products of the higher oxydation of hæmoglobin, containing larger proportions of oxygen. A body possessing the spectroscopic features of the so-called methæmoglobin is formed, as is well known, even without access of oxygen, by the action of very small quantities of acid on the hæmoglobin of arterial blood, and is produced at the exact moment when no more oxygen is given off to a vacuum. Since the addition of every reagent which produces the substance in question causes at the same time such a change in the hæmoglobin that no more oxygen can

* *Ber. d. deutsch. Chem. Gesell.* x. Jahrg. p. 692.

† *Zeitsch. f. Physiol. Chem.* Bd. i. p. 121.

be obtained from it by diminishing the pressure, but leaves it, on the other hand, in such a state that all chemical reducing agents convert it into ordinary reduced hæmoglobin, which then passes, in presence of fresh oxygen, into ordinary oxyhæmoglobin, it becomes thus easy to explain that the pretended synthesis or reconstruction of hæmoglobin out of hæmatin and globin has no other actual foundation than that which lies in the behaviour of the so-called methæmoglobin. This has indeed been already stated by others, and I have been accustomed, for many years, to explain it in this way in my lectures. From this it becomes, moreover, clear that the body in question can be nothing else than the more stable chemical compound of oxygen and hæmoglobin, which cannot be made unstable or dissociated by mere diminution of pressure, and which can only be decomposed or reduced by means of chemical action. Among the agents which convert hæmoglobin (the operation being of course carried out in presence of air) into the substance in question, that is to say, into the substance which alone may be properly called oxyhæmoglobin, I found trypsin particularly efficient; and since the further action of trypsin which leads to the formation of hæmatin and peptone starts only from this new body, I had particular reasons, in my short communication on the action of trypsin, in which I kept in mind its action on hæmoglobin, not to make any more definite statements as to whether I worked in presence of the atmosphere, a factor, on the necessity of which Hoppe-Seyler seems expressly to insist. Hoppe-Seyler is entirely unjustified in demanding from others that they should call only reduced hæmoglobin by the name hæmoglobin, as he does; this leads in his own case to numerous inconsistencies and obscurities of expression which least of all entitle him to make such an unnecessary remark, as that my statements are wrong; a remark which he appends to my observations on the digestion of hæmoglobin, which latter formed the starting-point of his further investigations (*vide loc. cit.*). It may also be remarked here that the formation of the real oxyhæmoglobin throws light at the same time on the vexed question of the ozonising action of oxygen in the blood, for it is ozone itself which produces this body. Both in a current of ozone and by the action of ozonised ether it may be obtained in the form of a body characterised by its beautiful brown glittering, exceedingly stable crystals. And such part of the oxygen of the blood as is converted into ozone, produces its oxydation product in the very first bearer of the ozone, viz., in the hæmoglobin itself: the hæmoglobin apparently decomposes of itself, because it, little by little, decomposes the loosely combined oxygen. Thus it is easily understood why other ozone reactions succeed so badly with blood: the hæmoglobin competes with the applied reagents.

Other pigments, such as those of the bile, fat, &c., as also most vegetable colouring-matters, fade slowly when exposed to light: whether this change is accompanied by accessory processes, such as oxydation, need not now be discussed.

The eye and the retina are characterised (putting aside the black pigments which are hardly destroyed by even the most energetic chemical reagents) by the presence in them of either the extremely sensitive visual-purple, or of other pigments which up to the present time have not been found to exhibit any marked relation to the light;

and the observation has previously been made* that the occurrence of the latter in coloured oil-drops bears a very close relation to the paucity or absence of visual-purple. The retinas of birds contain all the more visual-purple, in proportion as the number and intensity of colour of the oil-globules in the cones is small; in many birds, such as the pigeon and fowl, the retina is quite free from visual-purple, and in such cases the oil-globules are peculiarly well developed. The same appears to hold good for reptiles, as for instance in *Lacerta*, where there is no visual-purple, but much yellow pigment in the cones; in snakes we meet with neither the one nor the other.

It appears from all researches since the discovery of the retinal oil-globules by Hannover, that their colours are not to any great degree unstable. Alcohol, ether, etc., dissolve both the fat and colouring-matters. Heating up to boiling point, the action of alkalis and moderately strong acids, do not alter them, and in short it seems we have here to deal with exceedingly stable compounds. Now since these pigments occur without exception in globules which exhibit all the properties of fat-globules, and since this fat, as a solvent, might be something quite distinct from the, as yet, unknown but probably aqueous medium which contains the visual-purple in the rods, the question arose whether the three bright colours in the cones of the bird's retina, when freed from fat, and brought into aqueous solution, might not exhibit an amount of sensitiveness to light which should gain for them an importance similar to that of visual-purple. We were already aware that the bleaching of visual-purple is very much delayed by the absence of water, and we had observed that the "visual-yellow" acquired a high degree of indolence when it was kept dry or when it was fixed either in its natural substratum or by means of certain chemical compounds (corrosive sublimate) brought to bear upon it.

We supposed, therefore, that pigments naturally sensitive to light might exist in the anhydrous fats of the cone-globules, in a similar insensitive condition; and we might imagine that when the fats were in any way whatever partially decomposed or saponified, the cones which beyond doubt a refree from visual-purple might be provided with a substance of photo-chemical action, of whose existence there had been before no indication. This was one of the reasons which led us to separate the colour-substances of the retina of the bird from their natural com-

* Kühne u. Ewald, *Untersuch. Physiol. Inst. Heidelberg*, 1. Heft 1, p. 28.

bination with the fat; a second reason came from the wish to try, after removal of the solvent common to all, some new ones, in order to separate from each other the various kinds of colours. Neither result could be accomplished by anhydrous agents, such as the alcohols, ether, benzol, chloroform, bisulphide of carbon, etc., which had been hitherto used to dissolve fats.

Our first attempts to obtain coloured extracts from the retina of the bird with absolute or dilute glycerine, with bile, soap, or glycerine containing bile, were wholly in vain; with glycerine indeed the colouring-matters were often so evenly distributed that they seemed to have actually passed into solution; but when the fluid was filtered, only colourless drops went through. Thus the appearance of a solution, which was contradicted by the good preservation of the pigment-globules, for these, even after long action of glycerine in the dark, could be still distinctly perceived by the microscope, resulted from the equal distribution and refraction of the mass. After the failure of these first experiments nothing remained but the method of saponification, which seemed at first to promise little, but led, as will be shown, to a perfectly successful result.

From local reasons, we were led to use for these chemical experiments the eyes of fowls only, these being easily obtained in great numbers from the hotels of Heidelberg. The retinas of pigeons, which from the abundance of red cone-globules present in them would have better suited our purpose, were not to be had in the requisite numbers; so we used these only for microscopic investigations. The eye-ball was taken out of the fresh fowl's head, cleaned off on the outside, laid widely open in front, and the whole fundus immediately thrown, together with the retina after the escape of the vitreous humour, into absolute alcohol. As soon as 70 to 100 eyes had been collected in this manner, the yellowish alcohol was poured off, quickly evaporated on the water-bath, and the residue extracted with ether (which was mostly of a pale orange colour). This ethereal solution was added to the main solution obtained by treating the residue of the first alcoholic extraction directly with ether. Instead of ether, other solvents of fats, such as benzol, petroleum-ether, chloroform, etc., might have been used; but we found no occasion to depart from the method already begun, after we had convinced ourselves that none of the above-mentioned liquids extracted any further pigment from the residue already exhausted by ether, and after we had observed that each of the agents dissolved all the pigments together and not one more

than another. The same seems to hold good with cold alcohol, although the colouring-matters are dissolved by it only in a very small degree. This was to be expected, if the extraction depended essentially on the dissolving of fat; which, as is well known, is dissolved in any quantity by hot alcohol only.

The orange-red ethereal solution deposited, on evaporating, a fat of intense fiery-red colour, which we, according to the method of Heintz, saponified by dissolving it in a sufficient amount of boiling alcohol and adding a few drops of very concentrated caustic soda. In this process the colour scarcely changed at first, but became a lighter vermilion in proportion as the alcohol evaporated and the soap began to be separated, which event was much hastened by the addition of water. In order completely to remove the alcohol, boiling water was poured to the concentrated alcoholic solution of soap, and the mixture heated until all smell of alcohol had disappeared. In the first experiments we diluted the solution of soap so considerably, that it acquired after cooling, the consistence, at most, of thin soap-jelly, which was transparent and fiery-red in colour. When this was shaken up with ether, the latter turned to a deep orange-yellow, not reddish, while the soap below became a purer red, then of a cinnabar colour and at last rose-red, the ether apparently extracting chiefly yellow pigments, and only purple ones remaining dissolved or suspended in the soap. By extracting with fresh quantities of ether we were finally enabled to decant the last portions colourless, while at the line of junction of the two liquids there floated a powdery deposit of a deep rose-red colour, the solution of soap itself showing only a pale rose one. We were very unsuccessful in our attempts to isolate the rose-coloured substance, as the mass of ether deposit could not be filtered. When dried on the filter paper by evaporation of the ether and of the soap solution mixed with it, it gave up no colour to ether or to bisulphide of carbon, but imparted a faint rose tint to hot alcohol and to benzol, and also a very faint one to chloroform. Thus this process enabled us to extract orange or yellow pigments from the soap by means of ether, but proved to be inadequate to separate the purple ones, which were of particular interest, and besides failed entirely in consequence of the ether not separating properly from the solution of soap. After we had gained experience at the price of much precious material, the following correct mode of proceeding was found. The soap was prepared as already described but with a moderate excess of concentrated caustic soda, placed in the cold for twenty-four hours, separated by decanting in

the form of a solid cake from the completely colourless mother-liquor beneath, coarsely powdered and washed in cold water, until the excess of alkali was pretty well removed, *i. e.* until the wash-water just began to dissolve some of the soap, which could be seen from the appearance of a pale yellowish or reddish tinge in the filtrate. Thus purified the soap could be dried on the water-bath until it was capable of being scraped to thin shavings.

For dissolving the pigments out of the soap thus prepared, we can give the following formula, which we finally adopted after many experiments, which need not be related here. The powder is first shaken up with petroleum-ether, and after a few minutes filtered off. The residue in the filter is then washed off with ordinary ether, and shaken with ether as long as any colour continues to be given up. The first petroleum-ether solution is yellowish-green, from a colouring-matter which we may call Chlorophane. The second, or ether solution, is orange, or, when dilute, of a more pure yellow, due to a pigment which we may call Xanthophane. When the soap no longer colours the ether, it is of a fine red or purple, without any visible admixture of orange or yellow, *i. e.* neither vermilion nor cinnabar. If it is not of a pure purple colour, it is sure to give up more yellow pigment after standing a long time under ether, and in such cases we have found it expedient to wash with slightly warmed alcohol, which though it removes a little Rhodophane, as we may call the third pigment, takes up without failure the last remains of xanthophane. The purple soap now gives off to turpentine or to benzol a part of the rhodophane, so that clear solutions of a superb rose-colour are obtained; in order, however, to dissolve the whole of this pigment we know of no other method, than either to decompose the soap, or to dissolve it, either in hot water, or better in boiling alcohol, which give liquids of a deep purple. Bisulphide of carbon does not receive a trace of colour from this purple soap.

The solutions of chlorophane and of xanthophane are still in need of another purification, which is to be effected with some loss of substance by fractional solution, making use of the different solubility of the separate pigments in petroleum-ether. The chlorophane solution is evaporated to dryness, and the residue is then extracted with an insufficient quantity of petroleum-ether. The new solution indicates distinctly by its colour, which now passes more and more into green, the absence of the admixture of xanthophane, which remains undissolved with an orange-yellow hue. To purify the xanthophane, its ether solution is evaporated, and the residue washed with a little pe-

troleum-ether, under which process chlorophane passes into solution, and is removed, of course not without loss of xanthophane, a loss which is not made up by the quantity of xanthophane recovered from the first solution of chlorophane. The xanthophane free from chlorophane still holds some rhodophane, and in order to separate this also from it, the solid mass is treated with bisulphide of carbon, which leaves the rhodophane entangled in a gelatinous mass, while it dissolves the xanthophane with a deep orange-red colour. This solution, immediately and rapidly evaporated, dissolves in ether with a fine golden-yellow colour, leaving behind a small quantity of a dirty-looking deposit containing sulphur. In the last part of the operation special caution and very pure bisulphide of carbon are requisite, as the pigments easily decompose during the evaporation of this solvent, so that the ether used afterwards gives a filtrate which is sometimes turbid, but hardly or very doubtfully coloured. A rapid manipulation with frequent rinsing of the capsule, and very moderate heating, seem best calculated to ensure success. Aware of the possibility of the xanthophane decomposing during the purification, we did not neglect to control our estimate of its characters and reactions (these will be recorded farther on), by comparing them with those of xanthophane obtained directly by means of the petroleum-ether, for the latter being free from rhodophane, and being prepared without the application of bisulphide of carbon, might be considered as pure material. The rhodophane left behind by the bisulphide of carbon may of course still be made use of by drying and dissolving in turpentine, benzol, or hot alcohol.

As one and the same pigments in different solvents can form very differently coloured solutions, and this has been observed by Capranica in regard to the more permanent pigments of the retina, we have carefully noticed the appearance as well as the spectroscopic behaviour of the three pigments just mentioned, in the several solvents. The chlorophane dissolves in ordinary ether with the same greenish-yellow colour as in petroleum-ether, xanthophane in the latter as in the former, with the same tinge, varying from orange-yellow to pure yellow, and dependent upon dilution, and never becoming greenish-yellow. Bisulphide of carbon dissolves both with a deeper hue; chlorophane becoming more orange-yellow, xanthophane more reddish-orange. Thus the difference conspicuous in these substances, marked especially by the greenish tinge of the chlorophane, does not in any way depend on the nature of the solvent. The rhodophane, which, in the condition in which it has hitherto been obtained, does not dissolve at all in bisul-

phide of carbon, exhibited when dissolved in the three solvents which could be applied no difference of colour such as could be recognised by the naked eye, though the several spectra were in some respects different.

After we had succeeded in separating the pigments of the retina of the bird from each other, we were in hopes of clearing them also from other foreign admixtures, and perhaps of obtaining them in a crystalline form. This, however, we have not yet been able to do, as we found no process by which to remove from the pigments the impurities caused by the presence of slight quantities of soap or fatty acids. We at first followed the widely spread assumption that soaps are insoluble in benzol, bisulphide of carbon, etc., but this did not prove to be true; for we found, after evaporating the solutions of pigment, that all of the residues distinctly contained soap, as was shown by its appearing in a gelatinous condition before complete dryness was attained, as well as by the production of solid fatty acids and of oily drops after treatment with acids. This, after previous experiments which we had made on the saponification of the fatty tissues or of the yolk of eggs, surprised us less in the case of the ethereal solutions, than in the solutions prepared with petroleum-ether or with benzol, the latter of which, when used to extract the rhodophane, contained the greatest amount of soap. In using ordinary ether, which absorbs water to the same extent to which it is itself soluble in that fluid, we might indeed believe that it would absorb as much soap, not absolutely anhydrous, as would correspond to the water which it itself contained; but this explanation became worthless in the case of petroleum-ether, which seems to absorb no water at all. Petroleum-ether, in fact, appeared to dissolve soap, even when it was as free as possible from water, in which case no decomposition of the soap and passage of fatty acids into the ether (the necessary precursor of the soaps passing into solution) could have taken place. To clear up the matter we experimented on the colouring-matter of the yolks of eggs, first converting the alkali soaps into baryta compounds, and submitting the latter to the action of ether or benzole; but the results were not satisfactory enough to risk the material obtained with such great trouble from the fowl's eyes.

The pigments thus prepared therefore adhered to soap or even possibly were themselves compounds with alkalis. Though unable to decide as to the latter point, we think we should draw attention to the improbability of the hypothesis, for it would be necessary to assume that such alkali compounds were insoluble in water, in order to under-

stand why they cannot be extracted by heating with a little alkali or ammonia, either from the retina directly or from the fat obtained from the retina. As fats and fatty acids are soluble in concentrated acetic acid, it is easily understood why dried retinas also give up their pigments to this reagent and also why all mixtures of the soaps with the pigments obtained from the retina dissolve in glacial acetic acid without losing their characteristic colours. From such solutions water separates out the fatty acids, and to these the pigments always adhere. We have also dissolved the deeply coloured soaps in hot alcohol, treated them with a little glacial acetic acid (by which the colour was changed in one case only), and then diluted them with water. By this process the fatty acids were separated out, partly in crystals, partly in oily drops, but from these again the pigments could not be separated. Once attached by such a treatment to fatty acids instead of to soaps, the several pigments no longer showed any differences in solubility, since now the rhodophane easily dissolved in bisulphide of carbon, in ether and petroleum-ether, and xanthophane also rapidly dissolved in petroleum-ether, because the fatty acids soluble in all the above-mentioned agents now formed the vehicle for the pigments. The decomposition of the soaps by means of acids affords at all events a good method for preparing every kind of solution, even of rhodophane, of any degree of concentration. Rhodophane is then, when existing in a mixture with free fatty acids, soluble even in bisulphide of carbon, and when thus dissolved takes, in contrast with chlorophane and xanthophane, no other shade than the purple-red one characteristic of its other solvents. Dissolved with the soap in hot alcohol and treated with an excess of glacial acetic acid, we saw this beautiful colour gradually turn yellowish, and after 48 hours completely disappear, the decomposition taking place not more rapidly in light than in the dark.

Since the experience thus far gained admitted already of some physiological application, we for the present ceased from any further endeavours to obtain the retinal pigments in a state of perfect chemical purity. The mixtures of the pigment-soaps, for instance, would be exceedingly useful to prepare aqueous solutions and thus to decide the question raised as to the sensitiveness of these pigments to light. Even in the soap directly obtained from the fat, no notable tendency to undergo changes in light was to be observed, although we had in general made our preparations as much as possible in the absence of light, though not with such painful care as in working with "visual-purple." As, however, the deep colour of the rhodophane rendered the

chlorophane and xanthophane nearly imperceptible in the mixture, and whereas it was possible that these latter only were sensitive to light, they were also separately transferred into watery solutions. The quantity of soap which still adhered to them as an impurity was so small, that, with boiling water, no coloured liquids were to be obtained, and very slightly coloured ones with an excess of alkalies. Solution, however, was readily effected with a 5 per cent. bile salt solution, which absorbed all three pigments in considerable quantity. The solutions passed through the filter clear, only after standing about 24 hours. We did not find them more variable in light than those hitherto prepared with agents free from water. That which was most faded, after many days' exposure to the winter sun, was the chlorophane, then the xanthophane, whilst the rhodophane showed no change whatever, when we held it beside a specimen prepared in the dark for comparison. On the other hand, the solution of this substance in oil of turpentine is very inconstant, but not less so in the dark than in light, which is evidently to be explained by the fact that the turpentine becomes ozonised and thus bleaches the pigment. We must therefore answer our question, as to whether any products of the coloured fats of the bird's retina, if dissolved in another menstruum, would be sensitive to light after the fashion of the visual-purple, decidedly in the negative.

The mode of preparation of the pigments thus described renders inevitable a discussion as to their preexistence in the retina. A spectral analysis would of course afford the most conclusive means of deciding, but the apparatus was wanting, which would have enabled us to have carried out the necessary observations on the microscopic preparation of the retina with sufficient exactness; for instance with Browning's spectroscopic ocular, which Talma* used for this purpose, we could only succeed in obtaining very diffuse images, from which we did not feel confident to make statements regarding the spectra of the pigments contained in the separate coloured globules.

Those, who know the microscopic appearance of the bird's retina, cannot doubt that it contains at least three colours, one a ruby-red, another varying from an orange to pure yellow, and a third a greenish-yellow. It is therefore incomprehensible how any one could, contrary to all ocular demonstration, believe that appearances so different all arise from a single pigment. There is no need of dwelling on the fact that the eye often deceives us, since nobody doubts this, but it is dangerous

* *Onderzoekingen g. i. h. physiol. Lab. t. Utrecht*, Vol. III. II. p. 259.

to be blind to evidence of the kind which we have before us. If there were only ruby-red and orange fat-globules in the cones, it would indeed be credible, that the latter contained the same red substance as the former, but in a more dilute solution; but the hypothesis that the colour of the greenish-yellow cone-globules of the bird's retina arises also from the same single pigment, presupposes something which is impossible without fluorescence or dichroism, namely, that green of undoubted distinctness could arise by dilution from red or orange.

We dried preparations of the retina on cover-slips, and then treated them with alcohol, with ether, with benzol, etc., in order to see how the colours of the cone-globules changed while swelling up through dilution of the solution, and we convinced ourselves that even the largest and palest drops still allowed the three colours to be distinguished, especially when the drops lay close together, or at the moment when they ran into each other; we should even be inclined to maintain, that the difference between the pale yellow drops, formed by dilution from the original orange-coloured ones, and some of the slightly enlarged, intensely green-yellow ones became more distinct by this process than before. On the other hand, we are of opinion that the ruby-red globules do not quite correspond with the appearance of rhodophane, but approach more to a pure red, than the isolated pigment does; which is, however, easily understood, if they contain in addition some xanthophane*. In the pigeon's retina, the rhodophane, contained in very minute particles, probably themselves consisting of fat, is seen lying scattered in the finest distribution in the inner limbs of many cones, and having there the same unmistakable rose-red or purple shade which the rhodophane prepared by us possesses. We know, therefore, how this body looks *in situ*, and in the state of finest distribution or of optical dilution: and even then it in no way passes into orange or greenish-yellow. Where the larger ruby-red globules are changed by proper solvents into larger drops in more dilute contents, there is no similarity between these and the orange-coloured globules, much less the greenish ones. Consequently the pigments prepared by us agree very exactly with the colours conspicuous and distinguishable in the fat-globules of the cones of the fresh retina. As to the existence of blue globules which some assume, we were unable, in the fowl, or pigeon, to see them when we took care to exclude deceptions arising from contrast,

* In the falcon Schwalbe found (*Handbuch der Ophthalmologie v. Graefe und Saemisch*, Vol. I. p. 414), in apparently orange-coloured globules, two colour-substances visibly separated, a light-green one encircling a red one.

or to obtain blue pigment in substance though we employed various modes of separation (*e.g.*, colourless fats were tried as solvents of the isolated pigments and soaps).

Further means of proving the identity of the extracted pigments with those existing, *in situ*, in the retina were furnished by the greenish-blue and blue reactions with iodine, discovered by Schwalbe. As long as the pigments remained combined with fat, we tried in vain to colour them after their removal from the retina by means of iodine, and therefore had occasion to enquire as to whether there was contained in the cone-globules, besides the coloured fats, a second substance to which Schwalbe's reactions could be referred. As colourless cone-globules are not tinged blue by iodine, and the intensity of the reaction appeared to be only dependent on the quantity of colouring-matter in the oil-drop, the hypothesis was scarcely probable, and, in fact, we did not succeed in producing on retinas, which had once given up their colour by extraction, any more colouration by means of iodine. In the case of the fresh retina, it is not altogether easy to show that all colour-globules, and especially the lighter ones, give the iodine reaction; it needs long treatment with an excess of the reagent. It is, accordingly, not surprising, that the mixture of all the retinal fats in which the pigment in the ether extract is dissolved, should become an obstacle to the proper action of the reagent.

We have tried the experiment with iodine solutions of the most different kinds: with alcoholic solutions, with aqueous iodide of potassium solutions, and with mixtures of both; but always to no effect. We do not doubt, however, that the reaction when properly applied would succeed, for with the isolated pigments we obtained it perfectly. Chlorophane and xanthophane give the greenish-blue colour best, whereas in the case of rhodophane it came out darker, but more dingy and somewhat greenish. The masses being always somewhat alkaline, on account of the admixture of a quantity of soap, it was found expedient, in the case of all three substances, to treat them first with a trace of acetic acid. From this it is seen, that the reaction of iodine affords further proof of the agreement of the extracted pigments with those in their natural condition in the retina, and it deserves to be particularly noticed, that the peculiarly dingy colour of the rhodophane, under the action of iodine, may be also recognised in the retina, if we call in mind the peculiar tint which the diffuse red pigment of the inner limbs of the cones acquires under the influence of iodine, in those regions of the pigeon's retina which are conspicuous by their red colour.

Nitric acid and concentrated sulphuric acid, according to Capranica's statement*, tinge the coloured globules, and the coloured fat extracted from them, dark greenish-blue or blue. We obtained the reaction with nitric acid which contained a little nitrous acid, from the separate pigments also, the weakest from rhodophane, which became only for a moment pale bluish-green, and soon lost its colour entirely; the reaction was stronger and less transient with xanthophane and chlorophane. With sulphuric acid there were similar differences, for the two latter pigments only became a purer blue, while the former, after appearing momentarily blue-black, became dark brown. In the retina the reaction with sulphuric acid is not seen so well, because its albuminoid substances become too dark, and the fat in general red, so that a mixture of colours results.

After the foregoing comparisons, the preexistence in the retina of the three substances found by us cannot well be any longer doubted, and it must also be admitted, that the coloured cone-globules contain pigments which decompose with very great difficulty, which are not to any appreciable extent sensitive to light, and which are stable, not only when dissolved in fats, but even under the influence of boiling heat as well as during the process of saponification by concentrated alkali.

If the bird's retina contains several very different pigments, as has been shown, the spectroscopic examination of a solution prepared so as to contain, besides the fat, all the pigments together, affords but slight prospects of exactly determining the quality of the light which passes through the coloured globules to the outer limbs of the cones. Hence we attach special value to the spectroscopic results, to be discussed in the following paragraphs, obtained from the separate pigments. We exhibit on Pl. IV. fig. 14, Pl. V. fig. 15, chiefly for the sake of contrast, the spectra of the mixed pigments combined with fat, and dissolved in ether and in bisulphide of carbon. These, and all the other spectra, were taken from observations which we made in clear day-light or sun-light, by aid of the heliostat, with proper exclusion of too intense sun-light, as well as of the parts of the spectrum not needed. We have not employed the usual artistic method of reproducing the absorption bands, because they seldom come out in print corresponding exactly to the drawings, and preferred in their stead the clearer and more accurate representation in the form of curves, now so much more used

* *Arch. f. Anat. u. Physiol.* 1877, Heft 3, p. 285.

in chemistry. We have attempted to reproduce as truly as possible the positions of the maximum absorption and of its increase and diminution, also the relative darkness of the separate bands, and the occurrence of diffuse absorption at the ends of the spectra. All the solutions being poured into a Hermann's hæmoscope, which allowed a range in the thickness of the layer examined from 0 to 35 mm., were placed before the slit, and examined in such a manner, that we could judge, as the layer slowly grew wider, which shadows first appeared, and which portions of the separate bands succeeded each other; and from these data the measurements of the heights and forms of the curves were determined. Where we believed, as, in the case of chlorophane, in the red or orange, that we might suppose the absorption to be small, tubes with flat closed ends, and from 10—20 ctm. in length, were used. As the hæmoscope had to receive liquids which dissolve pitch-cement, and for which it was not originally constructed, we used an instrument put together with a lime cement, and greased the sliding cylinders, when necessary, with glycerine instead of with fat.

The spectrum, Fig. 14, of the mixed pigments shows the second broad band considerably fainter than the first one beginning before F, and also corresponding to the prevalence of yellow, a strong absorption at the beginning of the violet, while Fig. 15 exhibits the bands pushed far towards the red end, and a total clearing up in the violet. This is something peculiar to the mixed solutions in carbon bisulphide, and agrees with the change of tints directly visible to the naked eye. The second band, Fig. 15, may, in a good light and sufficiently thin layer of fluid, be seen double, although the two shadows are then very faint.

Fig. 16 shows a feature of chlorophane, which holds good both for solutions in ordinary ether, and for those in petroleum-ether. These solutions let through much more violet, but less blue and far more green, than the mixture of the three pigments. The most concentrated solution that we could obtain, gave in a layer of 20 mm. thickness the spectrum represented in the drawing, but in a thickness 20 ctm. exhibited no absorption either in the red after A or anywhere before *b*.

Dissolved in carbon bisulphide, the chlorophane turned orange-yellow, the green becoming imperceptible, and the spectrum (Fig. 20) now showed more indigo and blue, the green being covered, up to F, by the first band. After evaporation of carbon bisulphide, and being again dissolved in ether, it again gave the spectrum, Fig. 16.

Xanthophane freed from chlorophane gave only one band (Fig. 17), beginning before F, and a strong shading of the violet, which began

already in the indigo; dissolved in carbon bisulphide (Fig. 21), much stronger absorption of the indigo and violet was visible, beginning in the blue, and a band beginning directly behind E; the colour of the solution approached very nearly to that of the spectral red from B to C. Xanthophane therefore is not altered by being simply dissolved in carbon bisulphide, for if, after evaporation of the carbon bisulphide, it was redissolved in ether, the previous spectrum (Fig. 17) was again obtained.

The solutions of rhodophane showed also only one band of absorption, but of considerable breadth, which, in the benzol solution, spread over E and F. In the solution in turpentine-oil, it began between *b* and F, and occupied chiefly the space between F and G (Fig. 19). The two spectra showed differences in the absorption of violet, and the solutions did not appear exactly alike to the eye, the benzol solution being a little lighter, and more rose-coloured.

It being impossible to obtain the spectra Figs. 14 and 15 of the mixed pigments by superposition of the separate spectra, we tried to reproduce from the purified pigments a mixed solution of the same character as the original one. After decomposition of the soaps with acids, this was practicable for all of the substances, especially for rhodophane when ether and bisulphide of carbon were used as solvents; but we soon gave up these attempts, when we saw that the experiment cost more material than the result would justify.

From the same author, to whom we are indebted for the attempt to derive the different colours of the bird's retina from a single pigment, comes the further daring assertion, that this single substance (which does not exist) is identical with the yellow pigment of the fat-globules in the epithelium of the frog's retina. We were able to obtain this body in sufficient quantities from the remainder of the frogs' eyes which had been used in the preparation of "visual-purple," for we had been careful to throw immediately into alcohol all choroids with their retinal epithelium, and also the posterior hemispheres carefully cleaned, if they were of no other use. Thus, as in the case of the birds' eyes, the yellow fat of several thousand frogs' eyes was obtained, and from this was procured a soap containing the yellow pigment.

We now always easily succeeded in freeing completely from all colouring substances the dilute solution of soap by agitating it with ether. We also obtained the pigment itself, at least much freer from soaps, and more in the form of hard crusts, which separated

out even before complete evaporation. The attempt to get it in a crystalline form was not successful, whatever solvent we used.

Schwalbe's iodine reaction, which Boll obtained on the fat-globules of the retinal epithelium of the frog, succeeded admirably with this pigment, also the colouring with ammonia and sulphuric acid. In order to ascertain with certainty how the retinal fat of the frog behaved in comparison with that of the fowl, we prepared a part of the soap obtained from the former in the same manner as we did that from the latter; that is to say, we first obtained it in a dry state, and then extracted it with petroleum-ether. By this solvent all the pigment could be separated out, so that the soap became entirely colourless, and the solution became of a pure yellow colour only, neither a greenish nor an orange or red tint being seen; this pigment, like that of the fowl, could be transferred with ease to ordinary ether.

In Fig. 1 is represented the spectrum of the solution of the retinal epithelium in ether, in Fig. 3 that of the purified pigment, which we will call lipochrine; the two spectra possessing, as is seen, great similarity. This is to be expected, if the fat contains only one pigment; and we must draw attention to this striking difference as compared to the appearances of the pigment-spectra, and of the fat-spectrum of the bird's retina. Although these spectra resemble each other, nevertheless certain differences exist, probably on account of the presence of fat, which possibly may be of great influence on the tint of the solutions. In all spectra of lipochrine, there is to be remarked a less absorption in the region of the first band in comparison with that of the second, a circumstance which only occurs with the chlorophane of the bird's retina in ethereal solutions, as in Fig. 16. Dissolved in carbon bisulphide, lipochrine gives the spectrum Fig. 6, which besides the marked displacement of the bands up to E, shows even in concentrated solutions a total transmission of the violet. If fat is dissolved, together with the pigment, in carbon bisulphide, the spectrum in Fig. 4 is obtained, which is again different, showing less violet, and the first band beginning shortly before E.

The fact already mentioned in the *Unters. a. d. Physiol. Inst. z. Heidelberg*, Heft 3, p. 289, that the rabbit, whose fat-tissue is very pale, also has, in the retinal epithelium, nearly colourless fat-globules, led us to compare the pigment of the lobed fatty bodies in the abdomen of the frog with the peculiar retinal pigment of this animal; and as the investigation was carried on by the same methods as before, and the results coincided completely with that recorded of the retinal epithe-

lium, we confine ourselves to giving the evidence shown by the spectra of Figs. 2 and 5, Figs. 3 and 4. This pigment too gave the previously mentioned reaction with iodine (although it is true no trace could be produced on the fat-cells of the tissue itself), and also the blue or green colouration with ammonia and sulphuric acid. In the spectra we beg to call attention to the very distinct and uniform difference in height of the two curves. This goes far to prove the identity of the pigments in these tissues, so very different in origin and function; that is, of the one occurring in the epithelial cells of the frog's retina, and the one distributed in the ordinary adipose tissue.

As the lobules of the fatty bodies fade considerably in several days when kept moist, and in a single day if exposed to the sun, so do also the solutions of their fat in alcohol or in ether and those of the lipochrine extracted from their soaps. Dissolved in bile, the pigment did not fade sooner; in a very dilute solution on a white ground, and in a thin layer, it lasted at the longest 2—3 hours, before the colour was entirely gone. This, however, was accomplished only in July, between 12 and 3 o'clock, under a maximum action of direct sunlight, at a temperature kept at 12° C. by syringing with water.

After this experiment further investigations on the yellow pigment of the fat of different animals offer great interest, and it would be especially important to know, whether there are mammals, in which fat-drops identical in colour with those of the fat of other regions occur in the retinal epithelium. In man, whose fat is known to be yellow, we utterly failed to discover distinct fat-drops in the retinal epithelium; also in the pig and cow, where we hoped to find pale retinal fat. The skin of frogs gives up to alcohol and ether yellowish-green fats, from which we obtained by saponification a substance not differing from lipochrine.

We need not further insist that it is absurd to believe that the yellow pigment of frog's fat is identical with the mixture of the three pigments of the bird's retina; but the question still remained for us to investigate, whether lipochrine was not identical with one of them, either with chlorophane or xanthophane, which it sometimes resembles in appearance. But its solutions are neither so greenish as those of chlorophane, nor so orange-yellow as those of xanthophane, if we may judge from liquids tolerably equally concentrated; in a solid form or powdered with the fat it is most similar to chlorophane, the green tint of which under the like circumstances is less pronounced than usual. It is only necessary, however, to compare the spectra, Figs. 3 and 6,

of lipochrine with those of chlorophane, to discover such vast differences, that every thought of identity of the two bodies must vanish. While the first band of chlorophane begins some distance behind F, the first one of lipochrine passes considerably beyond F; the second band of the former extends over G, while that of the other ceases long before G (cf. Figs. 3 and 16). In the solutions of bisulphide of carbon, a similar relation is observed with reference to the lines E, *b*, and F, between the two bands which are, however, then shifted towards the red (cf. Fig. 6 and Fig. 20).

A comparison between xanthophane and lipochrine is made impracticable from the fact that the spectra of the former are all one-banded; and to look upon lipochrine as a mixture of xanthophane and chlorophane would, apart from other reasons, be inadmissible because the methods of separation which succeed perfectly with the mixture of these bodies in the bird's retina, never, as already remarked, produce two pigments when applied to the adipose tissue or to the retinal epithelium of the frog.

Consequently, in all no less than four distinct colour-substances of great stability have been discovered in the retina, including the retinal epithelium, besides the black pigment, which needs not to be discussed here.

Already Thudichum* and others have pointed out the similarity between the pigment of the yellow animal fats and that of the yolk of hens' eggs and of the *corpora lutea*, and even that of many yellow parts of plants. We therefore subjected the yolk of eggs and the *corpora lutea*, after the methods followed in the case of the retina, to a brief investigation. Fig. 7 shows the spectrum of an extract obtained by agitating fresh yolk of egg with a little alcohol and much ether. It is similar, with regard to the two bands, to that of the frog's fat (Figs. 1 and 2), but at G a third faint band is observed in it, and a much less absorption of the violet. This third band, described and figured by Preyer†, and denied of late by others, is only to be brought to view in a good light and with a very narrow slit, after suitable adjustment of the thickness of the layer by means of the hæmoscope. Sometimes, however, the most careful experiment will not succeed in proving its presence; and this we were able to trace to the well-known differences, either individual or depending on the variety of hen, which appear in the colouring of the yolks. A third band at G, but moved towards the red, appeared

* *Centralblatt f. d. Med. Wissenschaft*, 1869, S. 1.

† *Die Blutkrystalle*, Jena, 1871, Taf. II. fig. 13.

more constantly in the solutions of bi-sulphide of carbon (Fig. 10), obtained from the residue resulting from the evaporation of the ether-solution of the yolk-fat. Both petroleum-ether and ordinary ether extracted from dry yolk-soap the entire pigment, the behaviour of which before the spectroscope is shown in Figs. 8, 9 and 11. The resemblance of these spectra of the purified substance to those of lipochrine is indeed somewhat remarkable; but we must draw attention to two essential differences presented by the ethereal solutions: the bands of the yolk-pigment begin, 1st, always close by F, while their commencement in the case of lipochrine falls without exception and with great distinctness in the space between *b* and F; and 2ndly, the absorption in the case of the egg-pigment is never fainter at the first band *a* than at the second, as is constantly the case with lipochrine. These differences seem to us of so great weight, and of themselves such an obstacle to an assumption of identity, that it hardly adds further weight to the argument to note that the spectra of the carbon bisulphide solutions exhibit such marked contrasts with regard to the diffuse absorption of the violet end, as those represented in Figs. 6 and 11.

In our efforts to obtain from the yolk-soap by fractional extractions several solutions distinct in colour or in behaviour before the spectroscope, we were as little successful as in the preparation of crystalline pigments. We were struck by the somewhat greater solubility of the amorphous mass in dilute caustic soda, perceptible in the bright yellow colouring of the filtrate, and presenting a strong contrast to the hardly noticeable tinge which the pigments before named gave up to alkalies. This difference, however, may result from admixtures difficult of removal. As already observed the production of yolk-soaps by means of baryta-compounds led to no other result than those arrived at by the method ordinarily pursued by us; indeed the baryta-soaps, dried to a powder, had the disadvantage of swelling up in ether and thus producing a mass extremely difficult to filter. The sensitiveness to light of the yolk-pigment was found by us to be, in the ethereal and alcoholic solutions, or after being taken up with bile, about the same as that of lipochrine, and therefore also greater than that of the stable pigments in the bird's retina.

In Figs. 12 and 13 we have added copies of the spectra of solutions of lutein from the saponified residue of ether-extracts of the *corpora lutea* of the cow. As is seen, the resemblance between the ethereal solution of this and that of the purified egg-yolk is nearly perfect; in the carbon bisulphide solution the resemblance does not exist, inas-

much as Fig. 13 alone shows a total clearing up in the violet. It is not permissible to refer this to various degrees of concentration, because the differences represented in the drawing were most distinct just at that moment, when each of the solutions allowed the two bands lying in the green and the blue to appear with equal distinctness. Dissolved in petroleum-ether lutein gives a spectrum which slightly differs from that of the ethereal solution, that is only in the position of the first band. In Fig. 12 it is denoted by the dotted curve.

The behaviour of egg-yolk and of lutein under strong ammonia and sulphuric acid has been long known. We succeeded, as did Capranica, in producing distinctly on lutein Schwalbe's iodine reaction, while the yolk-pigment became, with this reagent, simply a dirtier yellow.

As none of all the pigments here discussed has yet been prepared in a state of perfect chemical purity, exact speculations as to their chemical affinity must for the present be withheld. We know that in the case of lutein, individual reactions and relations of solubility led to its being confounded with *bilirubin*, and to so great an error in the doctrine of the colouring substances of bile, that much work and trouble were necessary to clear the matter up. And even though the chemical knowledge of the substances confounded had only begun to be developed, that error would never have been committed, had differences like those here described between pigments been taken note of from the beginning. Without question the optical methods are of extraordinary exactness, and with substances which can only be obtained in very small quantities, they will be for a long time to come so essential that they will be with difficulty replaced. All the more caution is therefore necessary against underrating their results and suppressing differences which they exhibit where other methods apparently indicate resemblances only.

With reference to the cases before us, to ignore the results of spectral analysis would at once lead to ambiguities which might be said to be equivalent to the confounding of hæmoglobine with the so-called picrocarmine; the appeal to single chemical reactions, against many less conspicuous spectral differences, would only add to the chances, as has unfortunately already happened, of believing pigments which are easily distinguishable to be one and the same.

We lay great stress on the possibility now proved of separating in the bird's retina alone three entirely distinct pigments, and on the marked differences in spectroscopic behaviour of the substances above treated of; for we believe that in so doing we shall best point out

for further investigation the road which above all others must in the interest of physiology be entered upon, if we wish to gain that knowledge of the absorption of light in the retina which is absolutely necessary for the progress of the theory of vision.

EXPLANATION OF FIGURES.

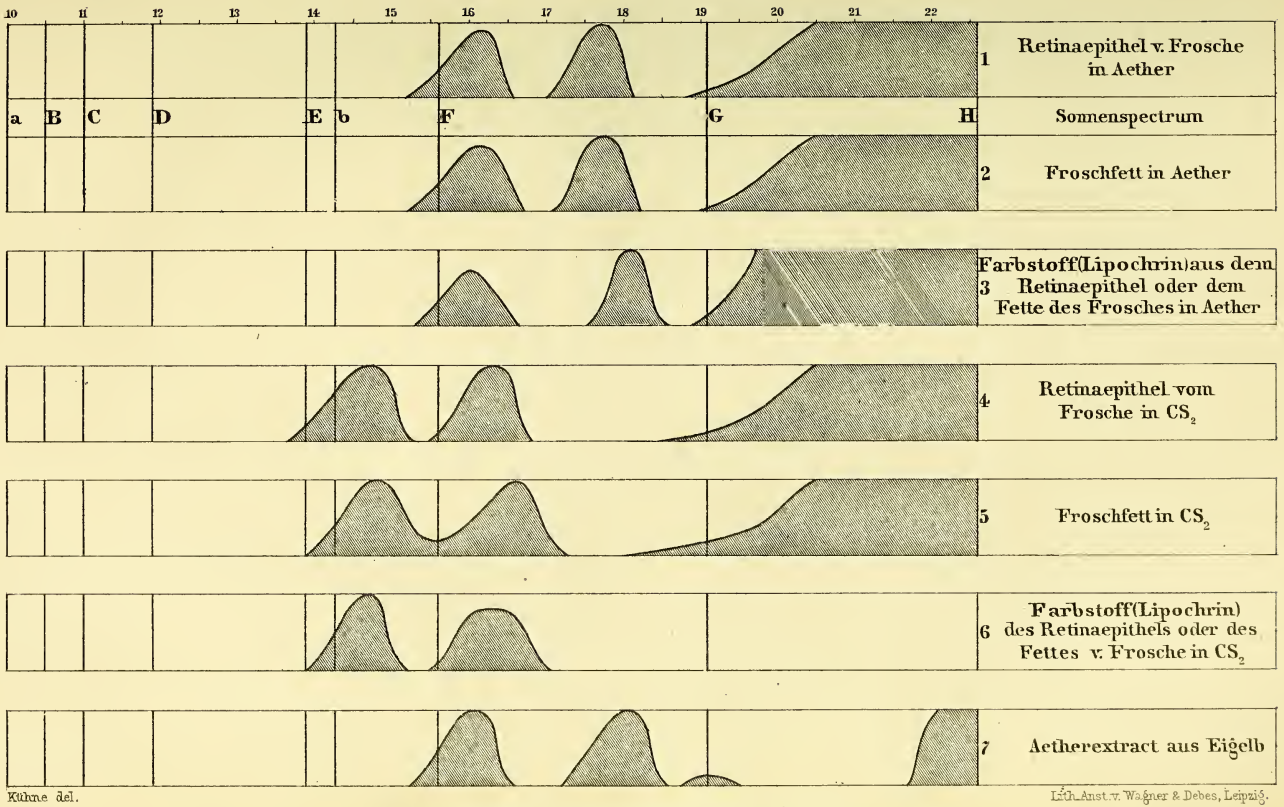
Pl. III. IV. V.

The drawings begin everywhere with Frauenhofer's line *a*, since in the red from A to *a*, no absorptions require to be indicated.

Pl. III. Fig. 1. Retinal epithelium of the frog in ether. Fig. 2. Fat of frog in ether. Fig. 3. Pigment (lipochrine) of retinal epithelium or of adipose tissue of the frog in ether. Fig. 4. Retinal epithelium of the frog in carbon-bisulphide. Fig. 5. Fat of frog in carbon-bisulphide. Fig. 6. Pigment (lipochrine) of retinal epithelium or of fat of frog in carbon-bisulphide. Fig. 7. Ether extract of yolk of egg.

Pl. IV. Fig. 8. Yolk of egg (free from fat) in ether. Fig. 9. Yolk of egg (free from fat) in petroleum-ether. Fig. 10. Yolk of egg in carbon-bisulphide. Fig. 11. Yolk of egg (free from fat) in carbon-bisulphide. Fig. 12, 13. Lutein in ether. ... in petroleum-ether. Fig. 14. Ether extract of retina of fowl.

Pl. V. Fig. 15. Fat of the retina of the fowl in carbon. Fig. 16. Chlorophane in ether or in petroleum-ether. Fig. 17. Xanthophane in ether. Fig. 18. Rhodophane in benzol. Fig. 19. Rhodophane in oil of turpentine. Fig. 20. Chlorophane in carbon-bisulphide. Fig. 21. Xanthophane in carbon-bisulphide.



Kühne del.

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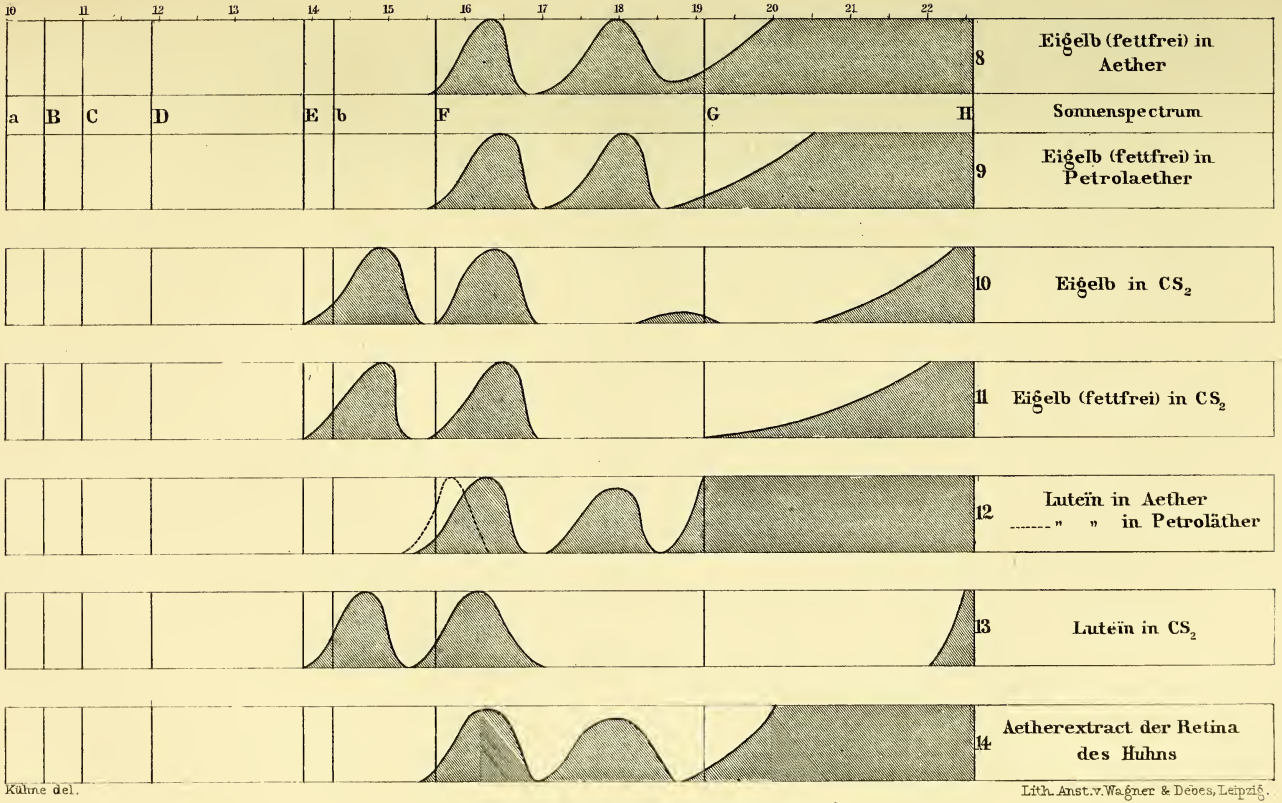

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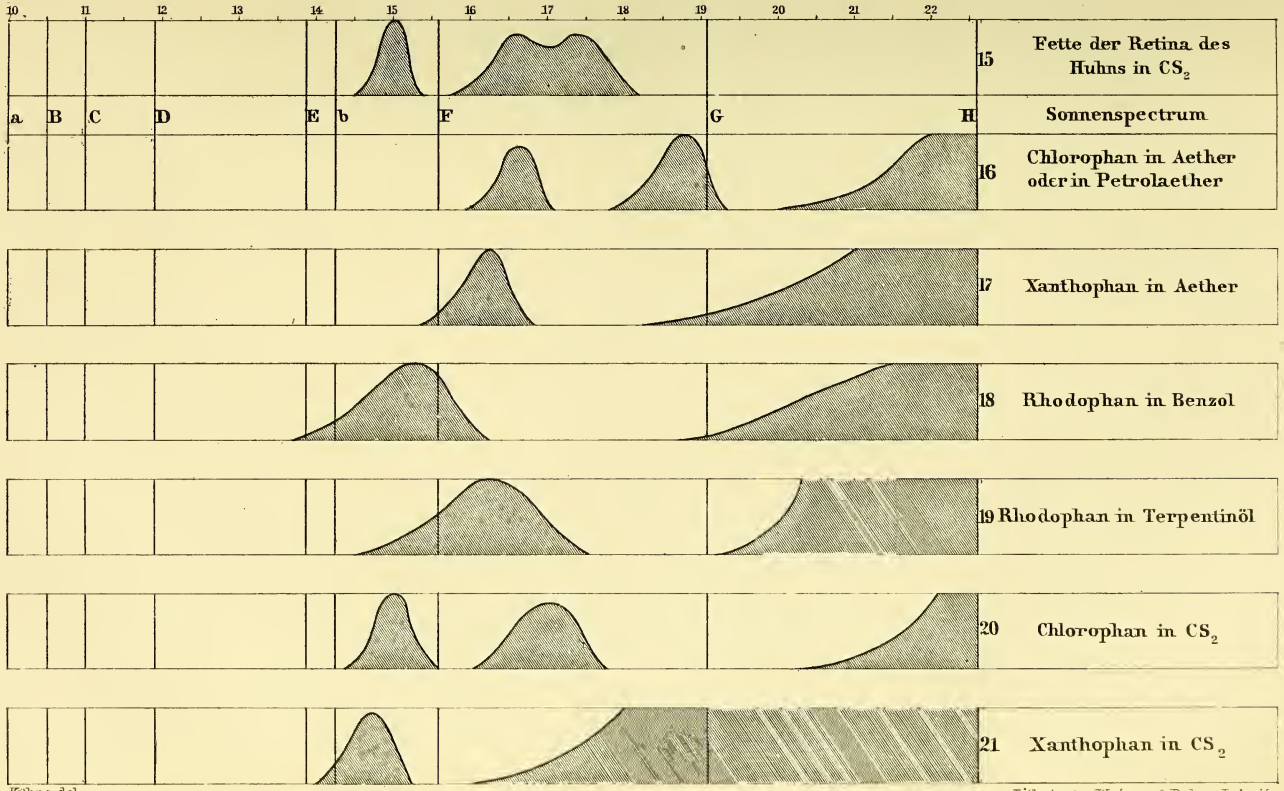

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ADDITION TO THE ARTICLE "ON THE STABLE COLOURS OF THE RETINA." By W. KÜHNE, M.D.,

Professor of Physiology in the University of Heidelberg.

FURTHER observations on the black animal-pigment mentioned at the beginning of the above article, have shown me that certainly the black granules and apparently the dark crystalline needles in the cells of the retinal epithelium are, under certain circumstances, not perfectly resistent to the action of light.

I noticed this as I was making observations on the sensitiveness to light of the coloured oil-globules in the retina of birds. Wishing to see how their colouring-matters would behave in the case of the cone-globules, when in their natural solution, I exposed fragments of a pigeon's retina to the action of light out of doors, in air-tight vessels, and examined them from time to time microscopically. For this purpose it was found most convenient to draw small pieces of the retina, moistened with various fluids, into moist chambers with flat capillary spaces, made by Geissler according to von Recklinghausen's plan, and then hermetically to seal up both ends of the tubes of these chambers. In other cases I sealed up the preparations in ordinary glass tubes, and examined them after breaking the tubes when they had been sufficiently exposed to light.

In order to protect them from putrefaction, whose progress might, in some unknown way, be influenced by the action of light, I used as the fluid to be added to them, either a 0·2 per cent. solution of salicylic acid, or, in order to have an alkaline fluid, a ·5 per cent. solution of sodium carbonate containing finely divided thymol in suspension. In addition I sealed up some preparations with ·5 per cent. sodium chloride solution, and heated them from ten to fifteen minutes up to 100°C. Others were previously heated with the soda solution without addition of thymol. For comparison I always made two preparations of each, of which one was kept in the dark, the other exposed on a bright surface to the action of light out of doors. Since, during the time of my experiments (from the 22nd of March to the 16th of April), it was generally cold in Heidelberg, and there was much rain, I do not fear that I have been misled by any direct action of heat.

In those preparations which had been kept in the dark, I could

detect no alterations of the colouring-matters at all; on the other hand, I found after exposure to light for twenty-five days, changes not only in the colours of the globules in the cones, as I had expected, but also, to my astonishment, in the black pigment of the retinal epithelium. Of the latter I had intentionally enclosed in the preparations only very small fragments.

The changes, which up to the present time have only been observed in the alkaline preparations, were as follows. In those which had not been warmed and contained thymol, and in which the pigment-cells in some places were still quite distinct, although somewhat swollen, the nucleus even being readily recognized, the largest part of the small pigment-needles had become light brown, yellow, pale yellow, or colourless. In some cells which were spread out quite flat, and whose processes were pointing in all directions, I could see no dark parts at all, but only such as were yellow or devoid of colour. These faded or colourless objects possessed the same needle-like shape as the original pigment. As I was able to control these changes every day, it cannot be doubted that it is the black pigment which, by becoming bleached, gives rise to the above appearances. In the alkaline preparation which had been heated without thymol, and had become quite slimy, the pigment-cells were of course from the outset indistinguishable, and only the corresponding heaps and rows of the black pigment-needles could be seen. These appeared to me during the course of the exposure to light, not only to become paler, but also to change their shape, becoming more rounded, and semiliquid. After twenty days' exposure, there was not a single trace of colour to be seen.

The colours of the cone-globules disappeared more rapidly under the action of light in the alkaline than in the neutral or acid preparations, when these were sufficiently flat and thin and not rolled up; the former bleached in about eight days, while the latter took fourteen.

The yellowish-green globules were the first to bleach, the ruby-red were the last, the red colour previously changing, in a remarkable manner, into a purple at a time when it was still quite intense. This speaks greatly in favour of the rhodophane which they contain being mixed with the more readily bleached chlorophane or xanthophane.

In the retinas of living pigeons and fowls I have been unable either by exposing the animals for several hours to the sun, or by holding them for a long time behind coloured glasses illuminated by the sun, to produce any changes in the colours of the cone-globules, or in the pigment-needles of the epithelium.

If one considers the extremely wide-spread occurrence in the animal kingdom of the black pigment of the eye, and other similarly stable pigments, it is scarcely possible to repress the idea that these, in addition to visual-purple, also represent visual excitants, or so-called visual substances, and are intended to be decomposed by light during life, and to yield those substances which stimulate chemically the terminal apparatus of the visual organ. If it is allowable, as I pointed out a year ago, to consider the chemical irritability of those portions of the sensory apparatus which are influenced by visual excitants, to be as marked as it must be in the case of the epithelium of the olfactory mucous membrane in respect to traces of odoriferous substances (*e.g.* musk), then even a body which is as little affected by light as the black pigment of the retina, would suffice for the origination of a visual process. From the same point of view the reactions to light of many pigmented organisms, and animal elementary organisms (such as the pigment-cells of frogs, chameleons and fishes), would be more readily understood than they are now; we might even imagine organs which, as the result of the action of light, give rise to general rather than special sensations, supposing that there exist pigment-cells in connection with simple sensory nerves. Krukenberg has recently informed me from Trieste that he has found the colours in the retina of the cephalopods, first noticed by Krohn, in 1830, to be as little sensitive to light as I observed those of the visual organ in Astacus fluviatilis. But one cannot doubt that even this substance is up to a certain degree sensitive to light, perhaps as much so as the pigments of the bird's retina are; the sensitiveness might at the same time be even greater than that of the black pigment.

Now as I have convinced myself by prolonged observations, partly on myself, and partly on animals (which, as in the case of the rabbit, must make but little use of the cones in seeing, since in them the cones are feebly developed or perhaps entirely absent), that even after the disappearance of the visual-purple acute vision is still perfectly possible, I have come to the hypothesis that the visual-purple, which is the most unstable visual excitant known up to the present time, serves for the perception of feeble light, while the other pigments whose occurrence has been observed in the eye, serve for that of more intense light. The occurrence of colourless cones and (in albinos) of colourless epithelium, shows that in addition to the above, colourless visual excitants must also exist.

If my hypothesis should bear good fruit, as I hope it will do, it

would perhaps lose that element of danger which it now possesses: it presupposes, namely, that there occur in the living eye changes which are, with our present means of investigation, objectively imperceptible; and these changes (apart from the view that they may not occur at all) may pursue a course quite different from that which has been observed in the substances we have as yet studied. We see isolated rhodophane for instance changed slowly by light, so slowly that we cannot hope to watch any corresponding change in the living eye. The hypothesis also permits us to suppose that certain pigments, though their proper character is perhaps that of visual excitants, may, notwithstanding their great sensitiveness to light, be at the same time really coloured screens, allowing certain rays of a particular wave-length only to reach the photochemical apparatus, and excluding other rays.

In order to make the caution with which I accompany my hypothesis more striking, I must call attention to the remarkable circumstance that the pigments of the bird's retina which we have discovered are mixed in such a manner in the oil-globules that the colours in the cones represent exactly half the spectral colours, viz. from red to yellowish green; so that with their complementary colours they yield all the colours of the spectrum. If, as Hering says, every pair of complementary or opposing colours produces a sensation by an antithetic chemical process in each substance, then it seems very inviting to look in the coloured cone-globules for the localising mechanism for objective coloured light, as Max Schultze assumed to be the case. This does not exclude the importance at the same time of the above pigments as visual excitants; we have not, however, as yet been able to make out that rhodophane is more easily decomposed by green light, and xanthophane and chlorophane by blue, as would be the case if these substances served not only as absorbers, but also for the reception of the stimulus of objective colours. We have merely observed that the three pigments are always bleached in the above-mentioned (p. 190) order, best of all under blue, less under green, not at all under red glasses. The identity of the absorption of coloured light with its chemical action, which was found to be so striking in the case of visual-purple and visual-yellow, is evidently not to be regarded as a general law; it holds good as little for the above substances as it does for several other colouring matters, such as purpurin, bilirubin, &c., which have recently been examined with regard to this point in this Laboratory.

HEIDELBERG, *May 8th*, 1878.